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UTILIZATION OF FRUCTOSE BY HYDROGENOMONAS H 16 (PART I)

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G.Gottschalk, U.Eberhardt, and H.G.Schlegel*

Experiments on the utilization of fructose by chemolithotrophically grown cells of Hydrogenomonas strain H 16 showed oxidation of this sugar after a lag of 20 min. The fructose is metabolized over the Entner-Doudoroff pathway. During adaptation to fructose, the content of phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, and enzymes characteristic for the Entner-Doudoroff pathway increases in the cells. During the change from chemolithotrophic to organotrophic growth, with fructose as substrate, the activity of ribulose-diphosphate carboxylase is reduced by 75% within 2 hrs, but decreases only slowly during fructose passages. Thus, fructose-grown Hydrogenomonas H 16 is able to fix carbon dioxide over the Calvin cycle.

As facultative chemolithotrophic microorganisms, knallgas bacteria grow under an atmosphere of oxyhydrogen and carbon dioxide as well as on a number of organic substrates. Thus, the Hydrogenomonas strains H 1, H 16, and H 20 utilize glutamic acid, aspartic acid, and a number of organic acids (Bartha 1962; Wilde 1962). However, of all tested sugars, these bacteria utilize only fructose; no growth was observed on saccharose, glucose, galactose, mannose, arabinose, ribose, sorbose, xylose, and xylulose. This differentiates H 1, 16, and 20

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** Numbers in the margin indicate pagination in the original foreign text.

from other Hydrogenomonas species (H. facilis; H. ruhlandii, and H. carboxydovorans) which are able to oxidize glucose (Schatz and Bovell 1952; Packer and Vishniac 1955; Kistner 1953). We investigated the fructose metabolism of Hydrogenomonas H 16 to determine why this sugar is the only carbohydrate that can be degraded.

1. Materials and Methods

Hydrogenomonas H 16 was grown in a mineral salt solution under 10% CO_2, 10 - 30% O_2, and 80 - 60% H_2 under magnetic stirring (Schlegel, Kaltwasser, and Gottschalk 1961). For cultivation on fructose, a total amount of 0.5% fructose was added to the medium.

Turbidity measurements were made in an Eppendorf photometer (d = 1 cm; 436 mμ) on suitably diluted suspensions (below U* = 0.3).

The cell protein was determined according to the biuret method (La Rivière 1958; Schmidt, Liaaen Jensen, and Schlegel 1963).

The respiratory metabolism was measured in a Warburg apparatus (Model V) manufactured by the Braun Co., Melsungen.

Radioactivity measurements. Determination of radiocarbon, incorporated by the bacteria, was performed by precipitating the cells on membrane filters (Göttinger Membranfiltergesellschaft) and counting the specimens mounted on brass blocks (Schlegel and Lafferty 1961). The instruments used included a counter FH 49, a sample changer FH 448, and a time printer FH 449 manufactured by Friesecke & Hoepfner, Erlangen.

Preparation of cell-free extracts. The bacteria were cooled in a Hughes /96 press to -40°C and then extruded through narrow slits by means of a hydraulic

* U = unit = standard amount of enzyme.

press (Hughes 1951). The protein determination in the cell-free extract was made by the Bücher method (1947).

Enzyme activity. One unit (U) represents the amount of enzyme catalyzing the reaction of 1 µmol substrate/min; 1 mU is the thousandth part thereof.

Enzymes and substrates for the enzymatic determinations were procured from Boehringer & Soehne in Mannheim. Ribulose-1,5-diphosphate was prepared from ribose-5-phosphate and ATP* over phosphoribose isomerase and phosphoribulose kinase from spinach (Horecker, Hurwitz, and Weissbach 1956).

Chromatography. Separation of the alcohol extracts was made by two-dimensional chromatography on 2043a paper manufactured by the Schl. & Sch. Co., Einbeck. The chromatographing was done against the direction of the fiber, with N-propanol:ammonia:water (6:3:1) and, in direction of the fiber, with sec. butanol:formic acid:water (6:1:2) (Metzner 1962; Hirsch 1963). For the production of roentgenograms, the chromatograms were kept for 2 - 4 weeks in direct contact with Agfa X-ray film SSS Blue.

2. Determination of Enzyme Activities

Hexokinase**. The following mixture was prepared by pipetting:

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* Abbreviations used: ADP = adenosinediphosphate; ATP = adenosinetriphosphate; EDTA = ethylenediaminetetraacetate; FDP = fructosediphosphate; F-6-P = fructose-6-phosphate; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GDH = α-glycerophosphate dehydrogenase; G-6-P = glucose-6-phosphate; G-6-PDH = glucose-6-phosphate dehydrogenase; GSH = glutathione; KDPG = 2-keto-3-desoxy-6-phosphogluconic acid; LDH = lactate dehydrogenase; NAD = nicotinamide-adenine-dinucleotide; NADP = nicotinamide-adenine-dinucleotide phosphate; PEP = phosphoenolpyruvate; 6-PG = 6-phosphogluconic acid; PGI = phosphoglucose isomerase; PGK = 3-phosphoglycerate kinase; 3-PGS = 3-phosphoglyceric acid; PK = pyruvate kinase; RDPC = ribulosediphosphate carboxylase; RuDP = ribulosediphosphate; TEA = triethanolamine; TES = trichloroacetic acid; TIM = triosephosphate isomerase; Tris = tris-(hydroxymethyl)-aminomethane.

** Boehringer Informations, 1961.

1.20 ml TEA buffer* of pH 7.6; 1.20 ml glucose solution (100 mg/ml); 0.20 ml $MgCl_2$; 0.10 ml ATP, 0.20 ml NADP; 0.01 ml G-6-PDH (0.2 mg/ml), and 0.10 ml crude extract**.

Phosphofructose kinase (Ling, Byrne, and Lardy 1955). Batch: 2.30 ml TEA-buffer pH 8.0; 0.10 ml 0.02 m F-6-P; 0.20 ml ATP; 0.03 ml $MgCl_2$; 0.20 ml 0.1 m GSH; 0.05 ml $NADH_2$; 0.01 ml aldolase (2 mg/ml); 0.02 ml GDH/TIM (2 mg/ml) and 0.10 ml crude extract.

Fructose diphosphatase (Racker and Schroeder 1958). Batch: 2.50 ml 0.1 m Tris-buffer pH 8.8; 0.10 ml 0.02 m FDP; 0.10 ml EDTA 1.2%; 0.10 ml $MgCl_2$; 0.10 ml NADP; 0.02 ml G-6-PDH (0.2 mg/ml); 0.01 ml PGI (2 mg/ml) and 0.10 ml crude extract (1:20 dil.).

Phosphoglucose isomerase***. Batch: 2.50 ml TEA buffer pH 7.6; 0.10 ml 0.02 m F-6-P; 0.20 ml $MgCl_2$; 0.10 ml NADP; 0.01 ml G-6-PDH (1 mg/ml), and 0.10 ml crude extract.

Glucose-6-phosphate dehydrogenase****. Batch: 2.50 ml TEA buffer pH 7.6; /97 0.20 ml $MgCl_2$; 0.10 ml 0.025 m G-6-P; 0.10 ml NADP, and 0.10 ml crude extract.

Fructosediphosphate aldolase. Batch: 2.74 ml collidine buffer (0.056 m; pH 7.4; 3×10^{-4} m monoiodoacetate; 2×10^{-3} m FDP); 0.05 ml $NADH_2$; 0.01 ml GDH/TIM (2 mg/ml), and 0.20 ml crude extract.

Ribulosediphosphate carboxylase (Peterkofsky and Racker 1961; Gottschalk 1964). The following mixture was pipetted into a centrifuge glass: 0.05 ml 1 m

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* Unless stated differently, these solutions were used in the following concentrations for enzyme determination: TEA buffer = 0.05 m; $MgCl_2$ = 0.1 m; ATP, ADP, $NADH_2$, and NADP = 10 mg/ml; 3-PGS = 50 mg/ml.

** The crude extracts had a protein content of 8 - 14 mg/ml.

*** Boehringer Informations, 1961.

**** Boehringer Informations, 1961.

Tris-buffer pH 7.8; 0.01 ml 0.5 m $MgCl_2$; 0.03 ml 0.1 m cysteine, neutralized; 0.02 ml serum albumin (40 mg/ml); 0.09 ml water; 0.10 ml $NaH^{14}CO_3$ (30 µmol, 22,580 Ipm/µmol); 0.10 ml crude extract (1:10 dil.), and 0.10 ml RuDP (0.5 µmol). The glass was stoppered with a plug extending far into the tube, and was then incubated for 5 or 10 min at 25°C. The reaction was stopped with 0.50 ml 12% TES and the fixed radioactivity was counted on 0.10 ml samples.

Triosephosphate isomerase. Batch: 2.70 ml TEA buffer pH 7.6; 0.10 ml triosephosphate solution, Boehringer; 0.15 ml $NADH_2$; 0.01 ml GDH (10 mg/ml). After all dihydroxyacetonephosphate had been hydrogenated, 0.05 ml crude extract (1:20 dil.) was added.

Glyceraldehydephosphate dehydrogenase. Batch: 2.50 ml TEA buffer 7.6; 0.10 ml 3-PGS; 0.20 ml ATP; 0.05 ml 0.1 m cysteine, neutralized; 0.04 ml 0.5 m $MgSO_4$; 0.05 ml $NADH_2$; 0.01 ml PGK (2 mg/ml), and 0.05 ml crude extract (1:50 dil.).

Phosphoglycerate kinase. Batch: 2.50 ml TEA buffer pH 7.6; 0.05 ml EDTA (5 mg/ml); 0.05 ml $NADH_2$; 0.20 ml ATP; 0.10 ml 3-PGS; 0.04 ml 0.5 m $MgSO_4$; 0.02 ml GAPDH (5 mg/ml), and 0.05 ml crude extract (1:20 dil.).

Phosphoglycerate mutase. Batch: 2.50 ml TEA buffer pH 7.6; 0.15 ml 0.5 m $MgSO_4$ + 2 m KCl (1:1); 0.10 ml ADP; 0.07 ml $NADH_2$; 0.10 ml 3-PGS; 0.02 ml 2,3-PGS (2 mg/ml); 0.02 ml PK (2 mg/ml); 0.02 ml LDH (2 mg/ml); 0.01 ml enolase (10 mg/ml), and 0.05 ml crude extract (1:20 dil.).

Pyruvate kinase. Batch: 2.40 ml TEA buffer pH 7.6; 0.15 ml 0.5 m $MgSO_4$ + + 2 m KCl (1:1); 0.06 ml 0.12 m PEP; 0.20 ml ADP; 0.05 ml $NADH_2$; 0.02 ml LDH (2 mg/ml), and 0.10 ml crude extract.

3. Results

a) Oxidation of Fructose by Resting Cells of Hydrogenomonas H 16

Hydrogenomonas H 16 grew on fructose with a generation time of 3 hrs and thus reached a greater rate of growth than under chemolithotrophic conditions (generation time = 5 hrs). Cells suspended in buffer immediately oxidized fructose if they had been grown on this medium; of chemolithotrophically grown bacteria, the fructose was utilized only after a lag phase of about 20 min (Fig.1b). Also for the incorporation of C^{14} fructose, there was a lag in radiocarbon assimilation by chemolithotrophically grown cells (Fig.2). Crouch and Ramsey (1962) had found that Hydrogenomonas facilis is able to oxidize glucose immediately after chemolithotrophic culture but at a reduced rate.

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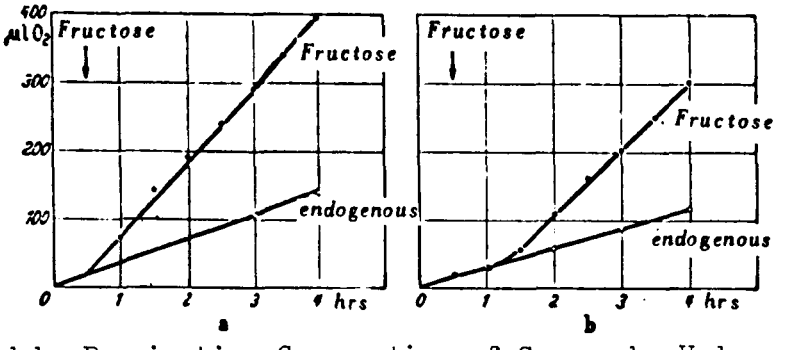


Fig.1a and b Respirative Consumption of Sugars by Hydrogenomonas H 16. a - Cells grown on fructose. 300 ml bacteria suspension (U = 0.48) were centrifuged off, washed twice with 0.066 m phosphate buffer of pH 7.0, and absorbed in 0.073 m phosphate buffer of pH 7.0. The suspension contained 0.50 mg protein/ml. The main space of the Warburg vessels was filled with 2 ml suspension by pipetting, while the central cylinder was supplied with 0.02 ml 20% KOH and the side arm with 0.2 ml 2% sugar solution. Q_{O_2} with fructose = 71 µltr/hr/mg protein (endogenous respiration deducted). An addition of L-arabinose, D-glucose, lactose, D-mannose, saccharose, and D-xylose did not increase the endogenous respiration. b - Chemolithotrophically grown cells. The experiments were conducted as given under a. The bacteria suspension contained 0.70 mg protein/ml. Q_{O_2} with fructose = 42 µltr/hr/mg protein (endogenous respiration deducted). An addition of other sugars did not increase the endogenous respiration.

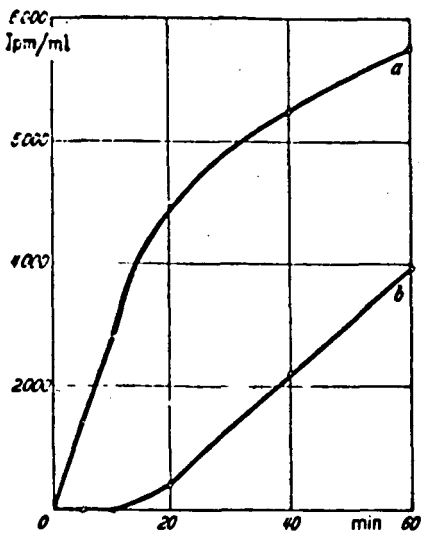


Fig.2 Incorporation of C^{14} Fructose by Hydrogenomonas H 16. 24-hour old cells, grown on fructose (a) or chemolithotrophically (b), were centrifuged off, washed, and suspended in a mineral nutrient solution without NH_4Cl. Samples of 20 ml suspension (U = 0.44) were shaken at 30°C. Each vessel was supplied with 10 mg of C^{14} fructose (5 μC) from which samples were taken after various intervals. After killing with 0.2 ml 70% $HClO_4$/ml suspension, the absorbed radioactivity was determined in membrane filters.

b) Formation of Poly-β-Hydroxybutyric Acid from Fructose

Hydrogenomonas H 16, in a mineral medium without nitrogen source, will form poly-β-hydroxybutyric acid from carbon dioxide under oxyhydrogen and from organic acids under air or oxyhydrogen, and then accumulates this substance /99 intracellularly up to 65% of the dry weight (Schlegel, Gottschalk, and Bartha 1961). A check was made whether the strain H 16 is similarly able to utilize fructose. In a fructose medium without nitrogen source, the turbidity of bacteria grown on fructose increased rectilinearly from the very beginning (Fig.3, Curve c). The PHBS content of the bacteria had increased as expected when the assimilated carbon is utilized exclusively for the synthesis of PHBS. In chemolithotrophically cultivated cells, the extinction increased only after

a longer lag phase (Fig.3, Curve a).

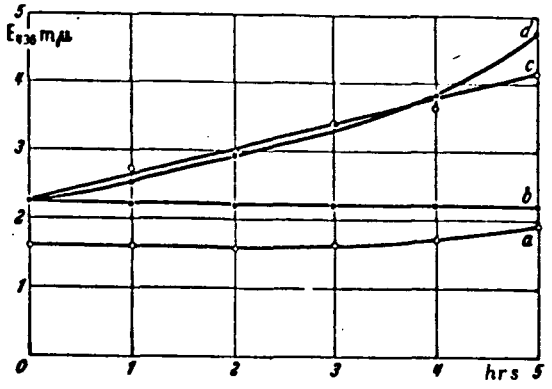


Fig.3 Formation of Poly-β-Hydroxybutyric Acid from Fructose by Hydrogenomonas H 16.
The bacteria, grown chemolithotrophically or on fructose, after washing with 0.066 m phosphate buffer of pH 7.0, were suspended in a mineral medium without nitrogen source. Samples of 20 ml each were shaken at 30° C in a 200-ml Erlenmeyer flask with the substrate to be investigated. a - Chemolithotrophically grown cells plus 0.5% fructose; b - Cells grown on fructose without substrate; c - Cells grown on fructose plus 0.5% fructose; d - Cells grown on fructose plus 0.5% sodium crotonate. In a and b, the PHBS content of the bacteria had not increased; in c, the content had increased by 0.31 mg/ml and in d, by 0.35 mg/ml.

c) Enzymes of Carbohydrate Metabolism in the H 16 Strain

The above-described experiments indicated that chemolithotrophically grown cells of Hydrogenomonas H 16 are not immediately able to utilize fructose. It should be of interest to determine the changes necessary in the enzyme apparatus of the bacteria for utilizing the fructose. Therefore, the activities of a number of enzymes from cells grown chemolithotrophically and on fructose were determined (see Table). The enzymes for decomposition of the C_3 compounds were present in sufficient amounts in both extracts. However, differences were observed in the glucose-6-phosphate dehydrogenase, in phosphoglucose isomerase, and in the aldolase. The results showed that fructose obviously is not decom-

posed over the Embden-Meyerhof pathway. The strong fructosediphosphatase and the weak aldolase activity indicated that a decomposition over the fructosediphosphate pathway seemed impossible. It must be assumed that the increase in phosphoglucose isomerase and in glucose-6-phosphate dehydrogenase has to do /100 with the fructose utilization. Since 6-phosphogluconic acid dehydrogenase could not be demonstrated, the decomposition of the 6-phosphogluconic acid could not have taken place over the pentosephosphates (Gunsalus, Horecker, and Wood 1955). Therefore, tests were made for checking on the decomposition pathway originally discovered by Entner and Doudoroff (1952) in Pseudomonas saccharophila, which leads over 6-phosphogluconic acid and 2-keto-3-desoxy-6-phosphogluconic acid.

TABLE

ENZYME ACTIVITIES IN HYDROGENOMONAS H 16

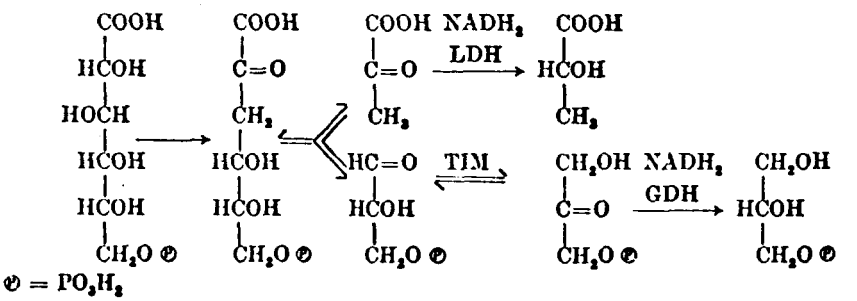
Enzyme	mU/mg Protein under $H_2 + O_2 + CO_2$	mU/mg Protein on Fructose
Hexokinase	12	19
Phosphofructose kinase	10	7
Fructose diphosphatase	350	110
Phosphoglucose isomerase	6	30
Glucose-6-phosphate dehydrogenase	2	36
6-Phosphogluconic acid dehydrogenase	0	0
Fructosediphosphate aldolase	14	4
Ribulosediphosphate carboxylase	29	8
Triosephosphate isomerase	670	590
Glyceraldehydephosphate dehydrogenase (NAD)	1540	625
Glyceraldehydephosphate dehydrogenase (NADP)	0	0
Phosphoglycerate kinase	320	620
Phosphoglycerate mutase	245	760
Pyruvate kinase	34	34

The bacteria, grown chemolithotrophically or on fructose, were disrupted on the Hughes Press (see Methods).

d) Demonstration of the Entner-Doudoroff Pathway in the Strain H 16

The enzymes 6-PG-dehydrase and KDPG-aldolase (McGee and Doudoroff 1954;

Kovachevich and Wood 1955), characteristic for this decomposition pathway, produce, from 6-phosphogluconic acid, equal amounts of pyroracemic acid and 3-phosphoglyceraldehyde. The following reactions formed the basis of the coupled enzyme test, made for demonstrating this enzyme system:



In an enzyme batch, containing 6-PG, LDH, $NADH_2$, and a cell-free extract /101 of bacteria grown on fructose, $NADH_2$ was utilized. If the same batch was mixed

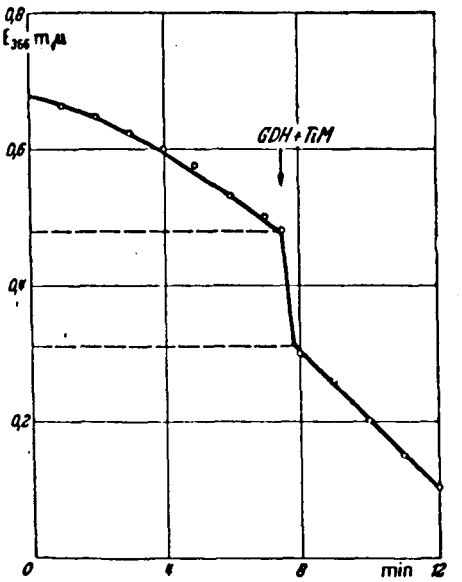


Fig.4 Demonstration of the 6-PG Dehydrase and the KDPH Aldolase in an Optical Test.
The cuvette (d = 1 cm) contained the following: 2.60 ml TEA buffer pH 7.6; 0.10 ml 0.1 m $MgCl_2$; 0.10 ml 0.025 m 6-PG; 0.05 ml $NADH_2$ (10 mg/ml); 0.02 ml LDH (2 mg/ml); 0.10 ml crude extract (8.7 mg prot./ml). After 450 sec, an amount of 0.02 ml GDH/TIM (2 mg/ml) was added.

with TIM and GDH, the extinction at 366 mμ immediately dropped by the amount by which it had previously been changed with LDH alone. Figure 4 shows a test performed in this manner. If, first, TIM + GDH and then LDH were added to the test batch, the extinction changed in the same manner. The activity of the Entner-Doudoroff system (6-PG dehydrase + KDPG aldolase) was 102 mU/mg protein for the strain H 16 grown on fructose. Cells grown chemolithotrophically, conversely, contained only 8 mU/mg protein.

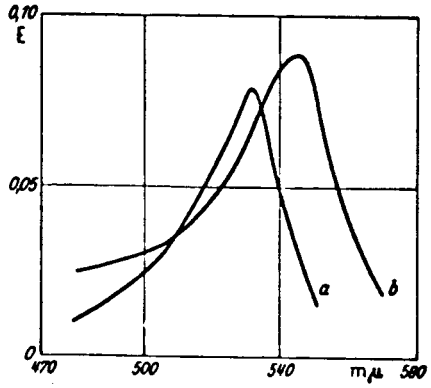


Fig.5 Absorption Spectra of Pigments Formed in the Periodate Oxidation of Desoxyribose (a) and of KDPG (b) with Thiobarbituric Acid.
2 ml TEA buffer of pH 7.6, 0.10 ml 0.1 m $MgCl_2$, 0.3 ml 0.025 m 6-PG, and 0.3 ml crude extract (12.9 mg prot./ml) of H 16 strain bacteria, grown on fructose, were incubated for 30 min at 25° C. After deproteinizing with TES, 0.25 ml 0.05 N sodium periodate solution in 0.05 N H_2SO_4 was added. After 30 min, the periodate excess was removed with 1 ml 2% sodium arsenite solution in 0.5 N HCl after which 1 ml of this solution was heated with 2 ml 2% thiobarbituric acid of pH 2, for 20 min in the boiling water bath.

According to Waravdekar and Saslaw (1957), the malondialdehyde, formed in the periodate oxidation of 2-desoxy sugars, will react with thiobarbituric acid to form a pigment with an absorption maximum at 532 mμ. From 2-keto-3-desoxyaldonic acids, formylpyroracemic acid is formed as the oxidation product; the pigment formed with thiobarbituric acid has an absorption maximum at

545 - 550 mμ (Weissbach and Hurwitz 1959). Figure 5 shows a sector of the absorption spectrum of the pigments, obtained with 2-desoxyribose and with the /102 deproteinized solution of a KDPG test batch. The position of the absorption maximum proved the existence of a 2-keto-3-desoxyaldonic acid in the test batch. The pigment was not formed if 6-PG or the cell-free extract were absent from the test batch.

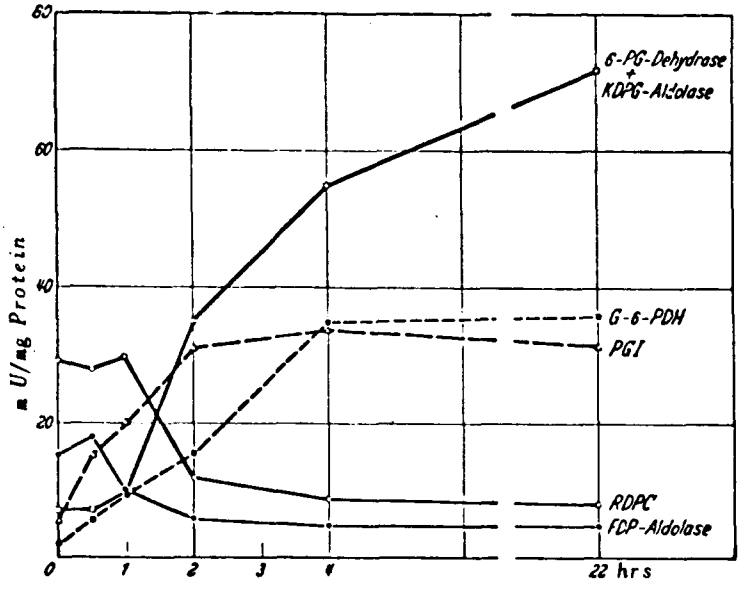


Fig.6 Variation in the Activity of Various Enzymes during Adaptation of the Strain H 16 to Fructose.
An amount of 6 ltr of a bacteria suspension was permitted to grow to an extinction of 0.38 under oxyhydrogen and carbon dioxide; 1000 ml were removed and 25 gm fructose were added to the remaining suspension, which was then stirred under air. After various intervals, 1-ltr samples were taken, centrifuged, washed, and frozen. From the bacteria, cell-free extracts were obtained in a Hughes press, for determining the activities of the indicated enzymes.

e) Induction of Enzymes Required for Fructose Utilization

During the adaptation of chemolithotrophically grown cells of the strain H 16 to fructose, the activity fluctuations of a number of enzymes were investigated. Fructose was added to a culture, grown under oxyhydrogen and carbon

dioxide; the culture vessel was washed with water and samples were taken at various intervals. The activities of several enzymes, found in the cell-free extracts, are plotted in Fig.6. After addition of fructose, formation of PGI and of G-6-PDH first set in. The synthesis of 6-PG dehydrase and of KDPG aldolase was accompanied by an inhibition of the RDPC and the FDP aldolase. The activity of these two enzymes dropped by 75% within 2 hrs but then remained constant over numerous generations. Only after diluting the chemolithotrophically grown inoculated material to $1:10^9$ did the RDPC activity drop to 6% of /103 that encountered in chemolithotrophic cells. Thus, bacteria, grown on fructose over three passages, were able to fix carbon dioxide in a fructose medium under air (Fig.7). The decrease in the rate of $^{14}CO_2$-fixation after several minutes was due to a dilution of the radioactive carbon dioxide with the CO_2 formed during oxidation of the fructose. That mostly autotrophic CO_2-fixation was involved here was demonstrated by a comparison of the CO_2-fixation products in bacteria grown chemolithotrophically and on fructose (Figs.8a and b).

4. Discussion

Knallgas bacteria have roused the interest of various research groups because of their chemolithotrophic metabolism. The changes in the metabolism of these microorganisms during growth on organic substrates have been little investigated, except for some papers on the degree of hydrogenase activity after heterotrophic culture of the bacteria (Kluyver and Manten 1942; Schatz and Bovell 1952; Atkinson 1954; Linday and Syrett 1958; Bartha 1962). In addition to a number of organic acids and amino acids, almost all of the described Hydrogenomonas species utilized glucose. Generally, no attempt was made to define whether fructose or other carbohydrates are also utilized. Packer and Vishniac (1955)

found that H. ruhlandii are able to grow also on mannose, galactose, and saccharose. The strains H 1, 16, and 20, investigated here, are unable to utilize glucose (Wilde 1962); we found, conversely, that they grow very well on fructose but do not utilize any other carbohydrate. This specificity for only one sugar is surprising in view of the structural similarity of the monosaccharides, although permeases and kinases, effective especially for fructose, were encountered in some bacteria (Eagon and Williams 1960; Palleroni, Contopoulou, and Doudoroff 1956).

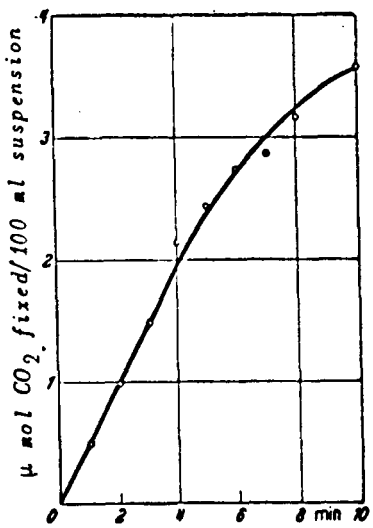


Fig.7 Incorporation of $^{14}CO_2$ by the Strain H 16, Grown on Fructose.
The bacteria were cultivated over three passages in a fructose medium (dilution of the chemolithotrophic inoculated material, 1:1.2 mill.) and then stirred in a mineral medium with 0.5% fructose without NH_4Cl at 30° C. To this, 100 µmol $NaH^{14}CO_3$ (0.3 mC) were added. The absorbed radioactivity in the samples, killed with 0.2 ml of 70% $HClO_4$/ml suspension, was determined on membrane filters.

During the rapid adaptation of chemolithotrophically cultivated bacteria /105 to fructose, the activities of the FDP aldolase, the phosphoglucose isomerase, and the G-6-P-dehydrogenase show distinct changes. The results demonstrate that

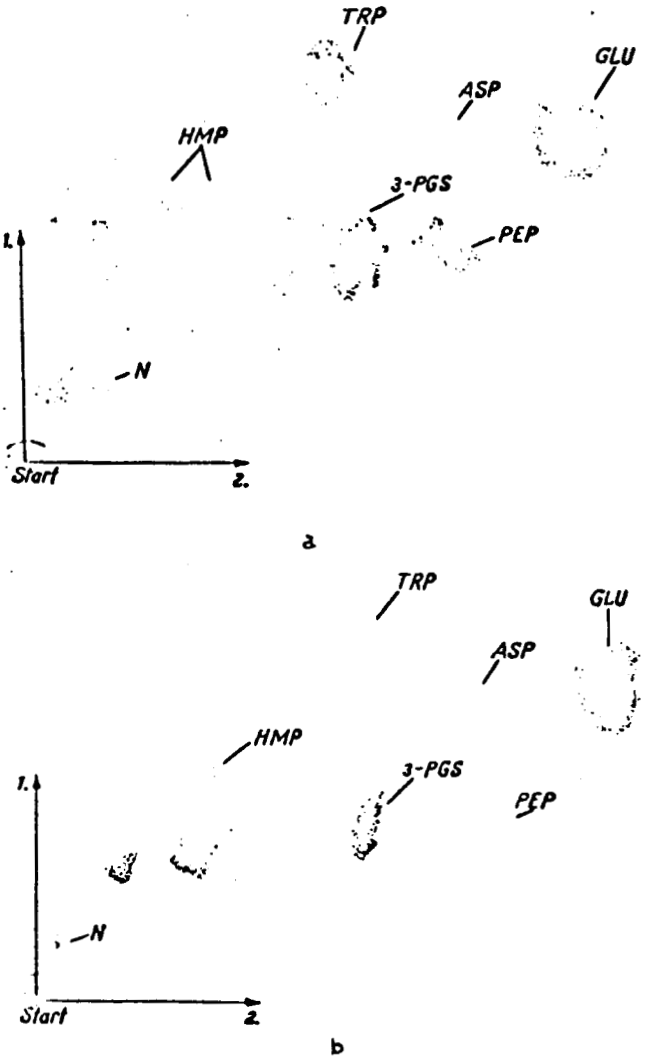


Fig.8a and b Radiochromatograms of Ethanol Extracts from Hydrogenomonas H 16 after Incorporation of $^{14}CO_2$.
a - Chemolithotrophically grown bacteria were suspended in a CO_2-free nutrient without NH_4Cl (U = 0.95). Under oxyhydrogen, 100 µmol $NaH^{14}CO_3$ (0.5 mC) were added to 20 ml of the suspension. After 90 sec, the $^{14}CO_2$ fixation was stopped with 20 ml boiling alcohol; the sample was centrifuged and the concentrated extract was two-dimensionally chromatographed (see "Methods"). Here, only the passage chromatograms are given; 55% of the fixed radioactivity was contained in the phosphate esters. b - Bacteria, grown over three passages on fructose, were suspended in 20 ml nutrient solution without NH_4Cl (U = 1.08). The suspension, stirred under air, was supplied with 100 µmol $NaH^{14}CO_3$ (0.5 mC) plus 2 mg fructose. After 90 sec, the samples were processed as under a. The phosphate esters contained 40% of the fixed radioactivity. The following abbreviations are used: ASP = aspartic acid, GLU = glutamic acid, HMP = hexosemonophosphate, N = nucleotides, PEP = phosphoenolpyroracemic acid, 3-PGS = 3-phosphoglyceric acid, TRP = triosephosphate.

the fructose is not decomposed by the strain H 16 over the Embden-Meyerhof pathway. The reaction sequence of triosephosphate → FDP → F-6-P, important in the autotrophic metabolism for the regeneration of the RuDP, is not reversible in the fructose utilization because of the presence of a strong FDP phosphotase and a weak phosphofructose kinase. Rather, the fructose enters the intermediary metabolism over the Entner-Doudoroff pathway; consequently, 6-phosphogluconic acid is irreversibly converted into 2-keto-3-desoxy-6-phosphogluconic acid which latter, in a reversible reaction, is split into pyroracemic acid and 3-phosphoglyceraldehyde. This catabolic pathway was discovered in 1952 by Entner and Doudoroff in Pseudomonas saccharophila and, in later years, encountered in a large number of microorganisms. Many Pseudomonas species metabolize carbohydrates over the Entner-Doudoroff pathway (de Ley 1960 and 1962) which also was encountered in representatives of the genera Xanthomonas, Agrobacterium, Rhizobium (Katznelson 1955 and 1958), Azotobacter (Kovachevich and Wood 1955; Johnson and Johnson 1961) and Gluconobacter (Stouthamer 1961), in Rhodopseudomonas spheroides (Szymona and Doudoroff 1960), in Pasteurella pestis (Mortlock 1962), in Streptococcus faecalis (Sokatch and Gunsalus 1957), and in Pseudomonas lindneri (Wang, Gilmour, and Cheldelin 1957). That species, belonging to the genus Hydrogenomonas, are able to make use of the Entner-Doudoroff pathway, had already been suspected by de Ley (1962) because of the fact that Pseudomonas saccharophila had originally been isolated as knallgas bacterium (Doudoroff 1940) and therefore is physiologically very similar to the described Hydrogenomonas strains.

During the growth on fructose, ribulosediphosphate carboxylase is further synthesized in part. Hirsch, Georgiev, and Schlegel (1963) found that the strain H 16 is able to fix CO_2 after cultivation on succinic acid. Hurlbert

(1963) reported that Chromatium and Thiopedia species, after cultivation on organic media, still contained 20 - 50% of the RDPC amount demonstratable in autotrophic cultures. Conversely, Santer and Vishniac (cited by Vishniac and Trudinger 1962) were able to demonstrate only 2% of the RDPC activity in /106 Hydrogenomonas ruhlandii and Thiobacillus novellus, if the bacteria had been grown organotrophically.

The fact that fructose is degraded by Hydrogenomonas H 16 over the Entner-Doudoroff pathway furnishes no explanation for the inability of this strain to utilize glucose. In the cell, G-6-P and F-6-P are in equilibrium over a strong phosphoglucose isomerase. Thus, either the conversion of glucose into G-6-P is impossible or else the glucose inhibits a certain reaction in the metabolism of the strain H 16. Experiments for settling these particular questions are reported in another paper.

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